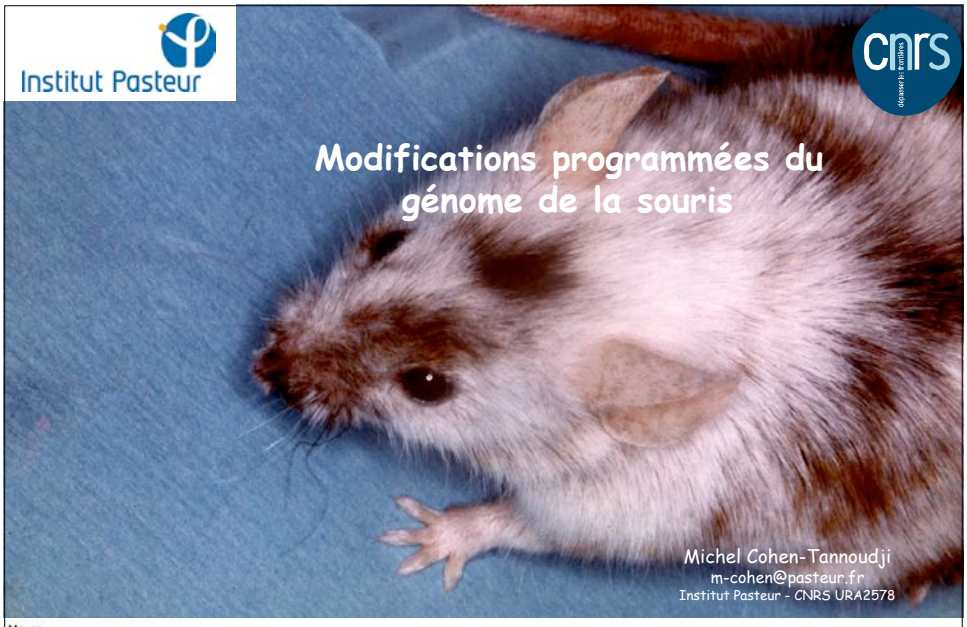

Modifications programmées du génome de la souris

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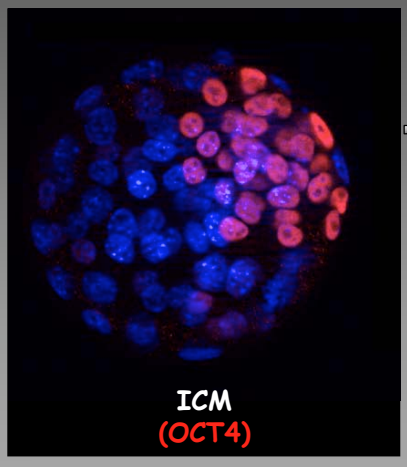
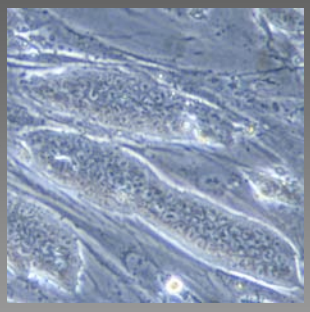
Mouse Functional Genetics Unit
Unité de Génétique Fonctionnelle de la Souris

http://www.pasteur.fr/recherche/biodev/

1981: mouse Embryonic Stem cell derivation

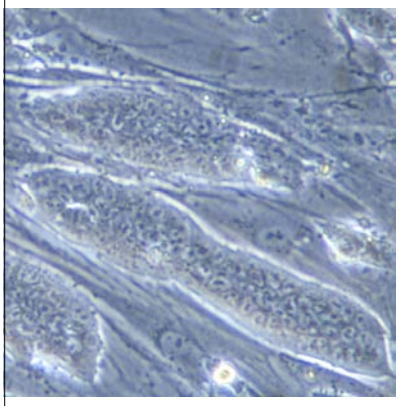




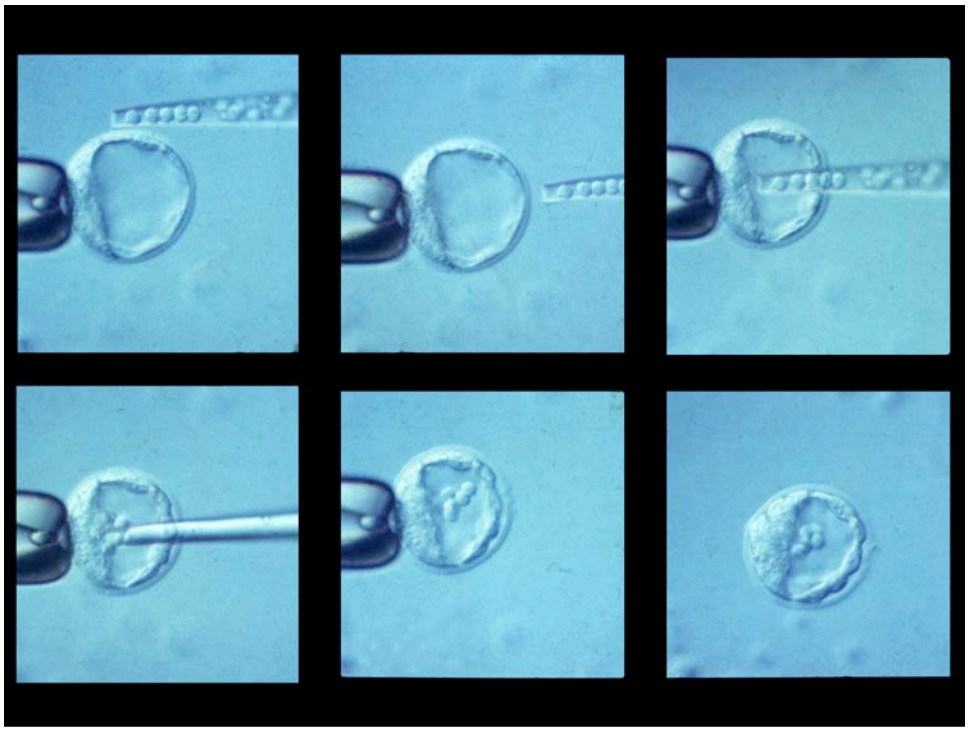
Establishment in culture of pluripotential cells from mouse embryos. M.J. Evans and M.H. Kaufman. Nature (1981), **292**, 154.

Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. G.R. Martin. P.N.A.S., (1981), **78**, 7634.

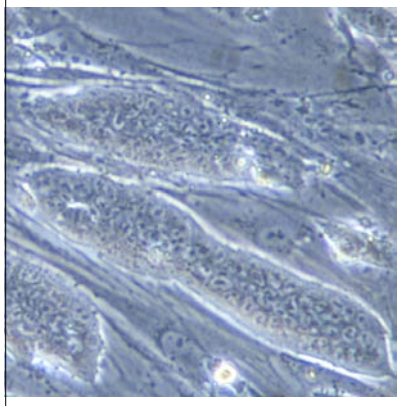
Embryonic Stem cells



- Originate from ICM/epiblast
- Stable diploid karyotype
- Clonogenic
- Unlimited self-renewal capacity
- Pluripotent: can generate all cell types
- Generation of germ-line chimeras : reshuffling of ES genome into the living animal



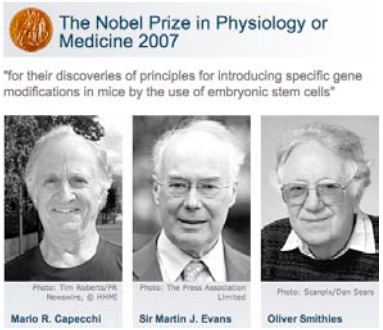
Embryonic Stem cells



- Originate from ICM/epiblast
- Stable diploid karyotype
- Clonogenic
- Unlimited self-renewal capacity
- Pluripotent: can generate all cell types
- Generation of germ-line chimeras : reshuffling of ES genome into the living animal
- Available in unlimited number :
  - Selection of desirable genetic modifications
  - Transfer of the genetic modification into the living animal

Gene targeting by homologous recombination in ES cells

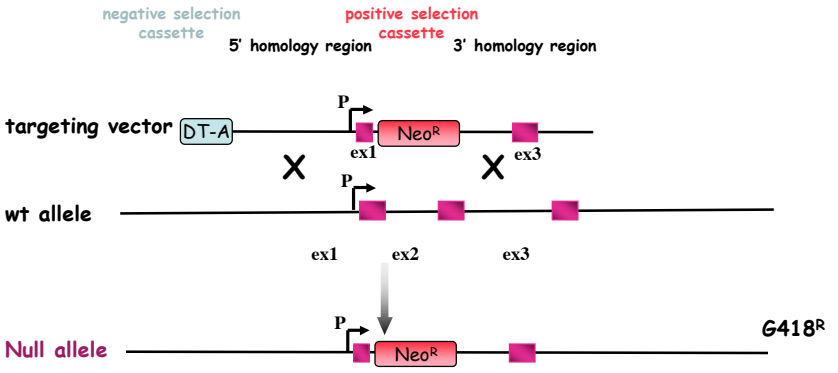
- Early 80s: Mario Capecchi and Oliver Smithies independently demonstate that homologous recombination can be used to target gene modifications in mammalian cells
- 1987-1988: targeted mutations in ES cells
- 1989: birth of the first knock-out mice



Gene targeting by homologous recombination in ES cells
Knock-out (KO)

Homologous recombination

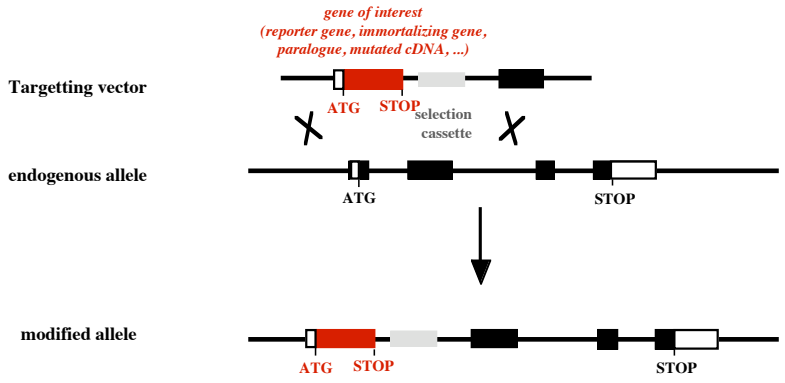
1 out of 10^6 to 10^8 electroporated cells



~ 1-10% G418^R clones

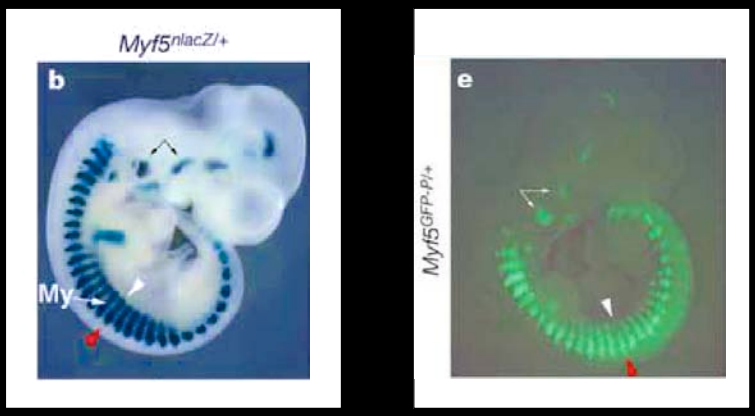
Knock-in: gene of interest expressed in place of the targeted gene

Classical transgenes (5-20 kb) are often lacking important regulatory elements



alternative approach: pronuclear injection of large transgene
(YAC or BAC)

« Knock-in » at the myogenic *myf5* locus

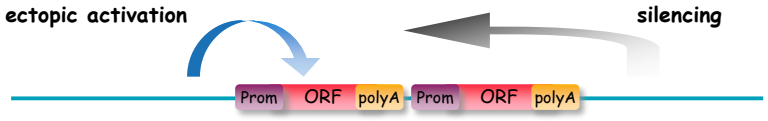


Kassar-Duchossoy *et al.*, 2004 *Nature* **431: 466-71**

Knock-in: targeted integration of a transgene into a permissive locus

Transgene expression is influenced by the sequences flanking the insertion site (« position effects »)

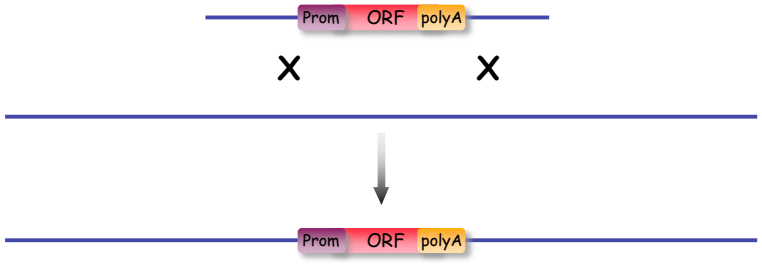
Random Integration



Inappropriate expression or absence of expression

Knock-in: targeted integration of a transgene into a permissive locus

targeted integration



Integration of a single copy into a permissive locus
widely expressed
little or no interference

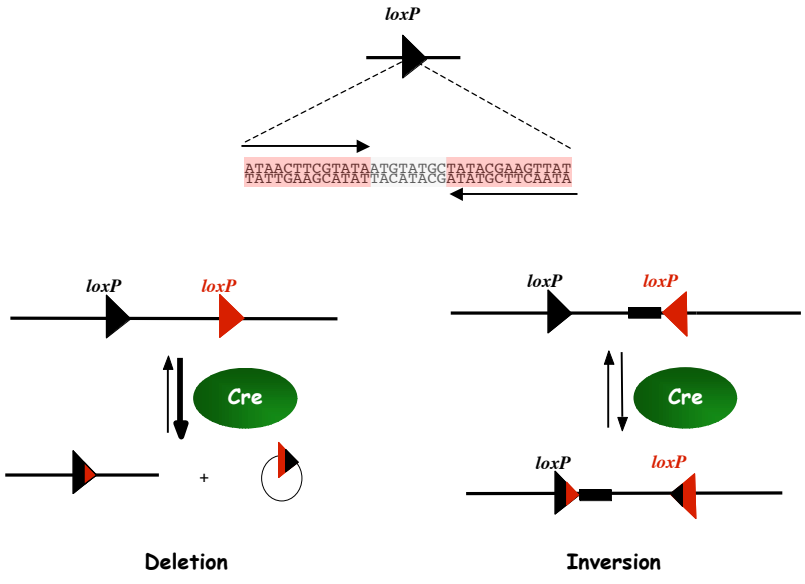
ex: *Hprt* or *Rosa26* loci

Conditional genome modifications

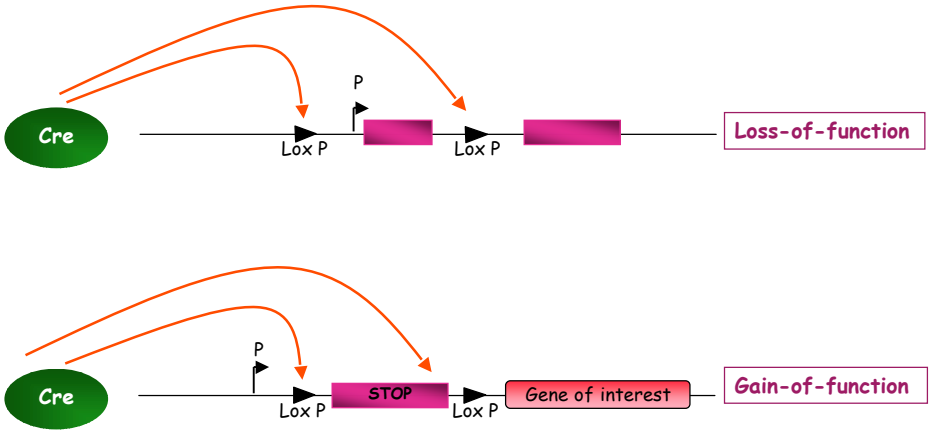
Site-specific recombinases (integrases)

- **Cre – loxP** *(bactériophage P1)*
- **Flp – Frt** *(Saccharomyces cerevisiae)*
- **Integrase *ΦC31* – attB/attP *(bactériophage ΦC31)***

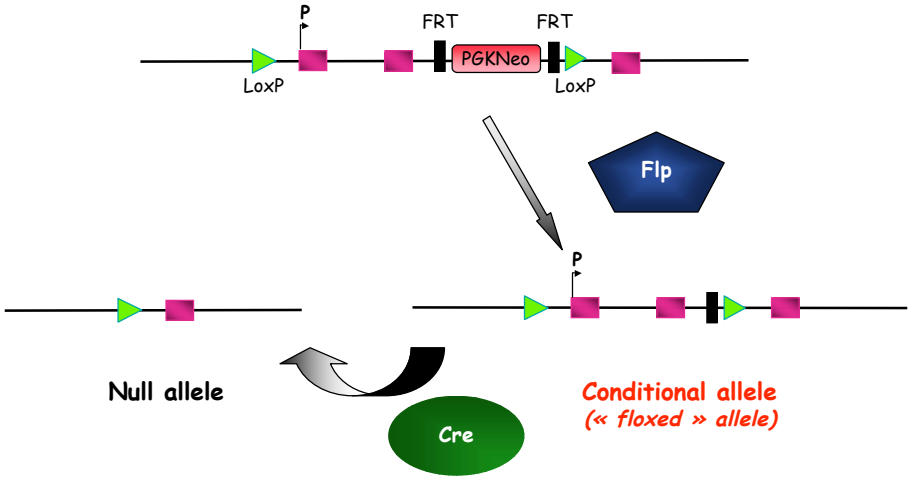
The Cre *loxP* system



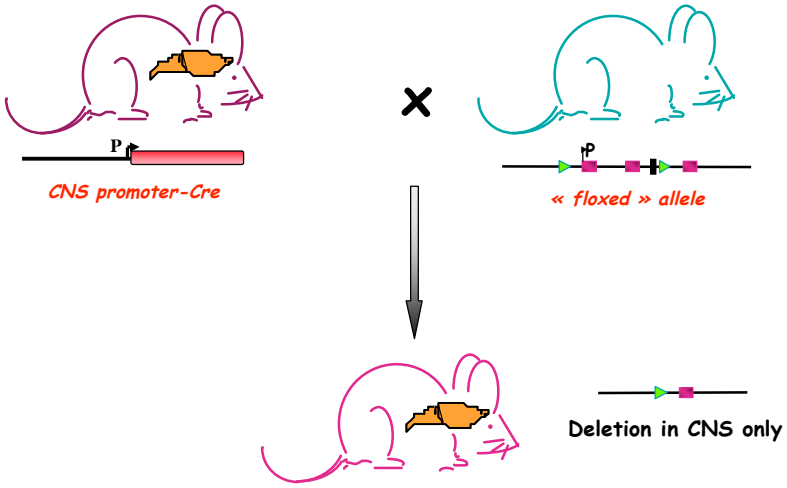
Conditional genome modifications



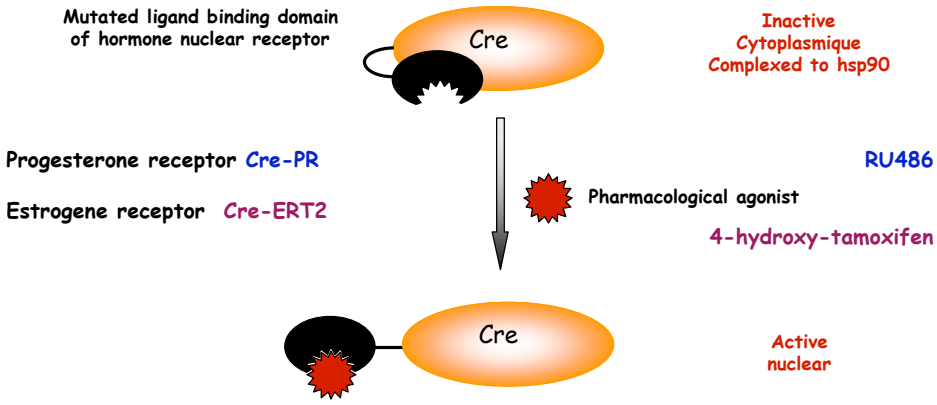
Combined use of Cre LoxP and Flp FRT for the creation of a conditional allele



Conditional mutagenesis
Cre recombinase

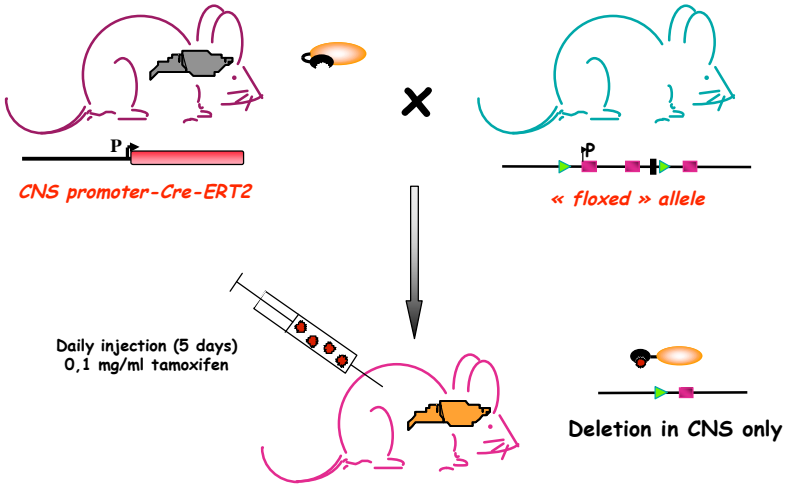


Spatial **and temporal** control of targeted mutagenesis
Inducible Cre recombinase



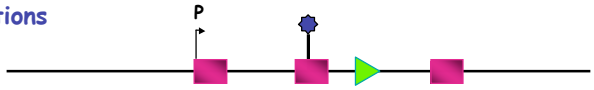
Spatial **and temporal** control of targeted mutagenesis

Inducible Cre recombinase

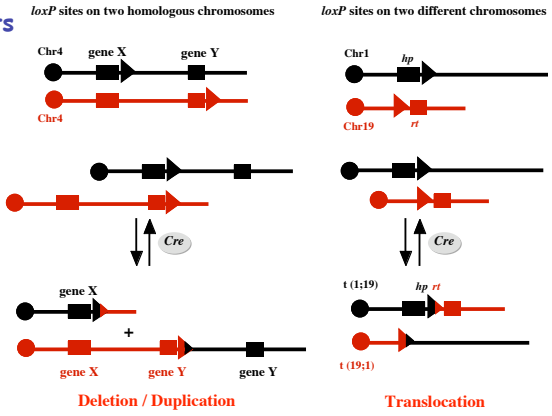


Other types of mutations

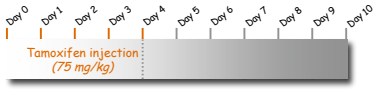
Subtle mutations



Chromosomal rearrangements



Acute gene inactivation in adult mice



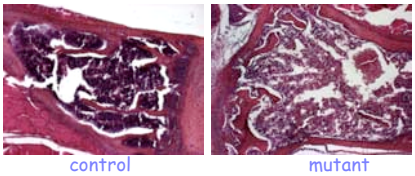
Experimental mice

mNle$^{flox/null}$ *RosaCre*^{ERT2}

Marie Lebouteiiler
Céline Souilhol

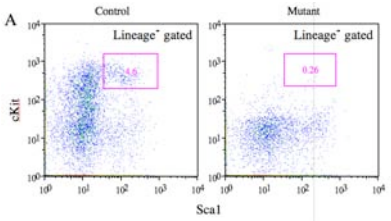
Bone marrow failure and rapid exhausion of HSCs

HES-stained sections of sternum



control mutant

FACS analysis



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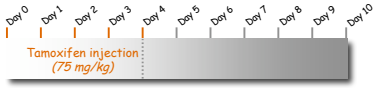


Experimental mice

mNle$^{flox/null}$ *RosaCre*^{ERT2}

Marie Lebouteiller
Céline Souilhol

What control ?

mNle$^{flox/null}$ *RosaCre*^{ERT2}

Identical genotype
Oil injection

mNle$^{flox/null}$

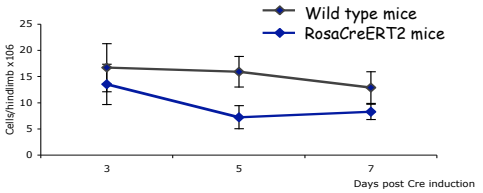
No CreERT2
Tam injection

mNle$^{wt/null}$ *RosaCre*^{ERT2}

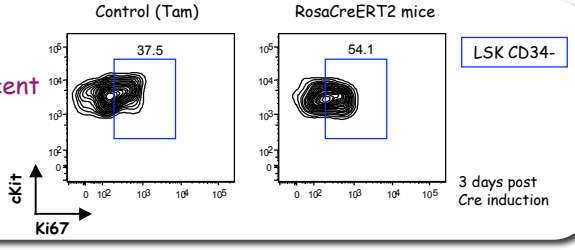
One WT allele
Tam injection

Cre activation affects various parameters of the hematopoietic system

Transient decrease in bone marrow cellularity



Modification of the quiescent state of HSCs



Marie Lebouteiller
Céline Souilhol

mNle$^{wt/null}$ RosaCre^{ERT2}

One WT allele
Tam injection

In vivo toxicity induced by high level of Cre activity
(pseudo loxP sites)

Stat5$^{flox/flox}$ RIP::Cre
Glucose intolerance

RIP::Cre
Glucose intolerance

Stat5$^{flox/flox}$ Pdx1::Cre
No glucose intolerance

TABLE 1
Studies that have used the RIP-Cre mice to inactivate genes targeted with loxP sites

N.D., not determined or the information was not available from the paper; N.T., not tested; JAX, Jackson Laboratory; MAM, Mark A. Magnuson; HE, Helena Edlund; RIP-Cre mice described in Ref. 13 were generated by Dr. Pedro Herrera.

Targeted gene	Glucose intolerance	Impaired insulin secretion	Cre control[a]	Source	Strain	Ref.
ARNT	Yes	Yes	No	MAM	N.D.	24
COUP-TFII	Yes	N.T.	No	MAM	129Sv/J; C57BL/6	10
PKCλ	Yes	Yes	No	MAM	N.D.	28
IRS2	Yes	N.T.	Yes	JAX	C57BL/6	17
HNF-4α	Yes	Yes	No[b]	MAM	CD1	22
SSTR5	Yes	Yes	N.D.	HE	N.D.	8
c-met	Yes	Yes	No	JAX	C57BL/6J	26
IRS2	Yes	N.T.	Yes	MAM	129Sv; C57BL/6	7
IRS2	Yes	N.T.	No	JAX	129Sv; C57BL/6	18
HNF-1β	Yes	Yes	No	MAM	129Sv; C57BL/6; DBA2	15
MEN1	N.T.	N.T.	No	MAM	N.D.	20
GR	N.T.	N.T.	No	Ref. 13	C57BL/6; CBAJ	13
Stat3	Yes	Yes	No	MAM	C57BL/6	14
Stat3	Yes	N.T.	No	MAM	129Sv; C57BL/6	16
MEN1	N.T.	N.T.	Yes	MAM	C57BL/6; FVB; 129Sv	12
Frataxin	Yes	Yes	Yes	MAM	>92.5% C57BL/6	21
Ca$_v$1.2	Yes	Yes	No[b]	MAM	C57BL/6	23
PPARγ	Yes	N.T.	Yes	MAM	129Sv; FVB/N; C57BL/6	27
IGF1R	Yes	Yes	No	MAM	129/Sv; C57BL/6; DBA-2	25
Foxa2	N.T.	No	No[b]	MAM	CD1	11
Tfam	Yes	N.T.	No	MAM	Mixed	9
InsR	Yes	Yes	Yes	MAM	129Sv; C57BL/6; DBA-2	19

[a] RIP-Cre only (no floxed alleles).
[b] Refs. 11, 22, and 23 included RIP-Cre mice with one floxed allele as control.

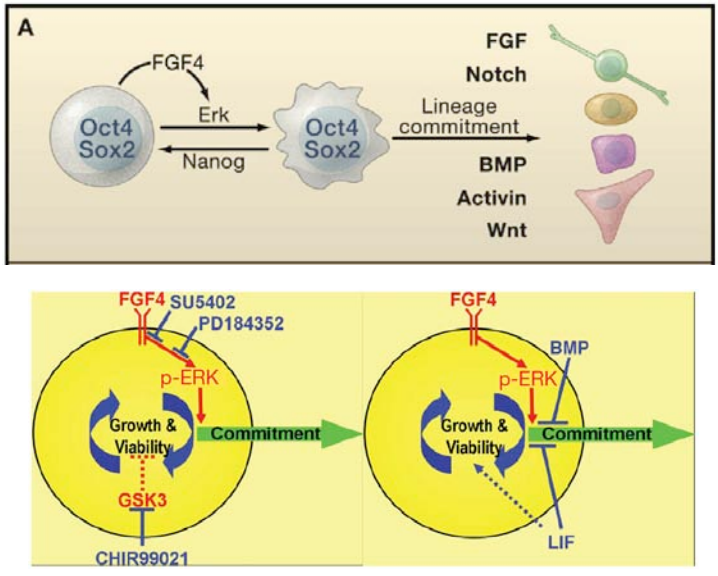
Lee *et al.*, 2006 *J. Biol Chem* **281: 2649-53**

Gene targeting in other mammals

Germ line competent embryonic pluripotent stem cells

- 1981-2008: only in mice!!!

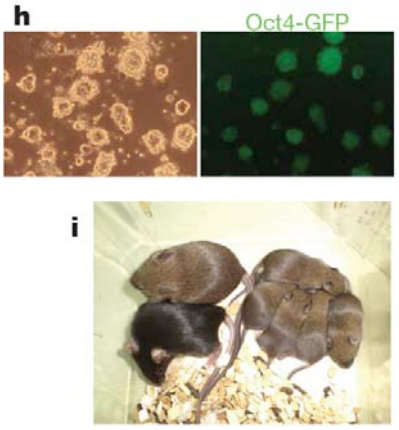
Capturing pluripotency by inhibitors



Yingi et al, Nature 2008

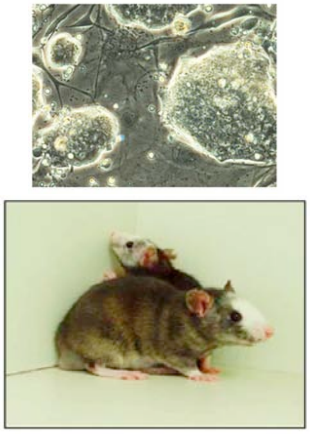
Capturing pluripotency by Fgf-Erk and GSK3 inhibitors

Mouse



Yingi et al, Nature 2008

Rat



Germ-line transmission
Stable transfection

Buehr et al, Cell 2008
Li et al, Cell 2008

Gene targeting in other mammals

Germ line competent embryonic pluripotent stem cells

- 1981-2008: only in mice!!!
- december 2008: in rat

Reprogramming of fœtal or adult somatic cells

- gene targeting is feasible in somatic cells
- reproductive cloning: nuclear transfer of somatic nuclei allows the production of genetically modified animals in larger mammals (cow, pig, sheep, …)